Occurrence of chrysanthemum downy mildew in Nara prefecture and its control with thermotherapy and fungicide

Kandai Yoshida, Shunsuke Asano and Yuka Sumikawa*

Abstract

In 2017, chrysanthemum downy mildew, which has not been reported in Japan since 1980, severely affected chrysanthemum production in Nara Prefecture, Japan. We investigated the occurrence of the disease at the fields in Nara Prefecture and the control efficacy of thermotherapy and fungicides against it. In the field survey, over 95 % of plants were diseased in seven cultivars, while no plants with the symptoms were observed in the other six cultivars. This suggests that some chrysanthemum cultivars had resistance to downy mildew. Chrysanthemum cuttings were treated with thermotherapy (treatment with hot water and hot air). The disease prevalence in both treatments was significantly lower than that in the non-treated. Severely diseased plants were sprayed with three fungicides (azoxystrobin, manzeb, and tolfenpyrad). While azoxystrobin and manzeb reduced the disease severity compared with non-treated, its efficacy was not so high. We concluded that the use of resistant cultivars and the application of thermotherapy can effectively control chrysanthemum downy mildew.

Key words: *Peronospora danica*, disease occurrence, hot water treatment, fungicide control

Introduction

Chrysanthemum morifolium Ramat is an economically important crop in Nara Prefecture. In 2016, the cultivated area was approximately 114 ha, with a production value of 1.4 billion yen (MAFF 2017). Small-flowered chrysanthemum is harvested from the field between May and December.

In Japan, *C. morifolium* with chrysanthemum downy mildew was first observed in Nagasaki Prefecture in May and June 1979 (Sakaguchi et al., 1980). After this record, the disease has not been reported in Japan for approximately forty years. However, it was recently recorded in Miyagi, Hiroshima, Hyogo and Osaka Prefectures in 2016 and 2017 (Yano personal communication, 2016; Yoshihara personal communication, 2016; Asano personal communication, 2017; Miyagi Prefecture, 2017). In Nara Prefecture, chrysanthemum downy mildew severely affected chrysanthemum production in 2017.

Chrysanthemum downy mildew is caused by *Peronospora danica* Gäumann, which belongs to the class Oomycetes. Symptoms include chlorotic or yellowish-brown spots on the upper surfaces of leaves and white or brown powdery

spots on the undersides of leaves under conditions of high humidity (Kishi, 1998). The pathogen has been reported on *Tanacetum cinerariifolium* Sch.Bip. in Japan (Ito, 1936).

Thermotherapy as a means of disease control may be applied to cuttings, fruits and seeds (Catherine and Regine, 1994). Hot water treatment is used commonly on rice seeds in Japan and effective in controlling rice blast and bakanae disease, and reduces chemical fungicide applications (Okabe and Baba, 2011). Hot water treatment is also effective in control of chrysanthemum white rust (Uchida et al., 1979; Hirayama et al., 2015; Yamagishi et al., 2016) although leaf burn occurs on some cultivars within a week post-treatment (Yamagishi et al., 2016). Thermotherapy with hot air has also proved effective in control of chrysanthemum white rust (Sugimura et al., 1996).

Because of the 40-year absence of *P. danica* from Japan, no published information on its control is available. In this study, we investigated the occurrence of chrysanthemum downy mildew in Nara Prefecture and evaluated the control efficacy of thermotherapy and fungicide applications.

Nara Prefecture Agricultural Research and Development Center, 130-1 Ikenouchi Sakurai Nara 633-0046, *Agriculture and Forestry Department, Nara Prefecture Govern, Nara, Nara 630-8501, Japan

Accepted February 13, 2019

Corresponding author: Kandai Yoshida (Tel: 0744-47-4481, E-mail: yoshida@naranougi.jp)

Materials and Methods

Occurrences of chrysanthemum downy mildew in field survey

We investigated the occurrence of downy mildew in 100 plants of a given cultivar at small-flowered chrysanthemum fields in Heguri town, Nara Prefecture on April 21, 2017. We observed symptoms in the lower, middle, and upper leaves in each cultivar.

Thermotherapy with hot water and hot air treatment

Infected mother chrysanthemum plants ‘Benichiyo’, ‘Hotaru’, ‘Jukou’ and ‘Ryuusei’ were collected from fields in Heguri town, Nara Prefecture in November 2016. Hot water treatments were applied on December 26, 2016. 8 cm cuttings were taken from mother plants with latent downy mildew, and soaked in a constant-temperature water bath (YS-501 Tiger Kawashima Co., Ltd) at 46°C for 3 min. After that, the cuttings were held under running tap water due to cool them. Hot air treatments were conducted according to Sugimura et al. (1996). Infected, potted mother plants of 10 cm height were brought into the incubator at the following temperatures: 20°C for 8 h, 35°C for 6 h, and 15°C for 10 h, between December 16 and 26, following which 8 cm cuttings were taken. For plants treated with both treatments, hot water treatment was conducted after hot air treatment according to the methods described above. After treatments, cuttings were rooted in plug trays (3.4 × 3.4 × 4.4 cm, 128 plugs/tray) in an non-heated greenhouse, with between 9–16 cuttings per treatment. A randomized complete block design with two replications was used.

We surveyed the occurrence of the disease, and the leaf burn on January 5, 12, 24 and 30 in 2017. Leaf burn was assessed using the following scale: 0–4: 0 = no leaf burn, 1 = slight brown spots on upper leaves, 2 = leaf burn occupied less than 10% of upper leaf area, 3 = leaf burn occupied more than 10% of upper leaf area or shoot tip was burnt, 4 = dying plants. The leaf burn rating was calculated using the formula: $(4A + 3B + 2C + D)/N$; A, B, C and D are the numbers of plants on scale 4, 3, 2 and 1, respectively, and N is the total number of plants. We used Ryan’s multiple test to assess differences in accumulation of disease prevalence, and the Steel–Dwass multiple range test to assess differences in disease severity, among the cultivars. Throughout this study, $p < 0.05$ indicated significant difference.

Fungicide treatment

A fungicide trial was conducted in an non-heated greenhouse in 2017. Diseased chrysanthemum seedlings of ‘Benichiyo’, ‘Ryuusei’, ‘Jukou’, and ‘Hotaru’ were collected from Heguri town, Nara Prefecture in November 2016, and planted in planters (60 × 17.5 × 18.5 cm). In April, plants with severely diseased lower-to-middle leaves were sprayed with fungicides three times at seven-day intervals between April 5 and 19. Three fungicides were sprayed at 20 L a⁻¹, diluted with tap water to the following concentrations: manzeb (Zimandaisen WP; Dow Agrosience, Japan) at 2.5 g L⁻¹, tolfenpyrad (Hachihachi EC; OAT Agriot, Japan) at 1 g L⁻¹, azoxystrobin (Amistar 20 FL; Syngenta Japan, Japan) at 0.5 g L⁻¹. Control plants were sprayed with water. We marked the uppermost symptomatic leaves and evaluated disease severity in the upper leaves than the mark. The disease severity was surveyed on April 12, 18, and 25. Each fungicide was applied to 8–17 plants per cultivar. A randomized complete block design with two replications was used. The disease was assessed on a scale of 0–5 in which: 0 = no symptoms; 1 = >5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–70%; 5 = symptoms occupied more than 70% of leaf areas. A disease severity was calculated using the formula: $(5A + 4B + 3C + 2D + E)/N$; A, B, C, D and E are the numbers of plants on scale 5, 4, 3, 2 and 1, respectively and N is the total number of plants. Protective value was calculated using the formula: $\left\{ \frac{X-Y}{X} \right\} \times 100$, in which X = disease index for non-treated plants, Y = disease index for treated plants. We used the Steel–Dwass multiple range test to assess the effects of the fungicide treatments on disease severity based on the daily assessments.

Results

Occurrence of chrysanthemum downy mildew in field survey

The disease prevalence and symptoms are presented in Table 1. Six cultivars showed no symptoms, while the remaining seven cultivars, with the exception of ‘Irodori’, were over 95% infected (Fig. 1c, d). We observed that, predominantly, lower to middle leaves turned yellowish brown; and white powdery mold on the undersides of lower leaves in diseased plants (Fig. 1a, b). Disease severity differed among the cultivars. Lower to middle leaves died in ‘Hakutaka’ and lower to upper leaves and/or plants died in ‘Jukou’ and ‘Shiragiri’.

Table 1. Disease prevalence and symptoms for chrysanthemum downy mildew in Heguri town, Nara Prefecture on April 21, 2017

Fields	Cultivars	Percentage ^z diseased plants	Symptoms
A	Jukou	100	lower to upper leaves died or plants died
	Irodori	100	lower to middle leaves turned brown
	Seikanata	0	
	Unknown	0	
B	Kiyohime	95	lower to middle leaves turned brown
	Shiragiri	97	lower to middle leaves turned brown
	Seikanata	0	
	Akane	0	
	Haruki	0	
C	Syuumokuba	100	lower to middle leaves turned brown
	Kyoumaru	100	lower to middle leaves turned brown
	Shiragiri	100	lower to upper leaves died or plants died
	Hakutaka	100	lower to middle leaves died
	Irodori	12	lower to middle leaves turned brown
	Seikanata	0	
	Kawakaze	0	
	Natumaru	0	
D	Benihikari	0	
	Kiyohime	100	lower to middle leaves turned brown
	Unknown	0	

^z Disease prevalence was recorded for 100 plants of a given cultivar in each field.

Efficacy of thermotherapy against chrysanthemum downy mildew

In the control (non-treated) plants, symptoms appeared on January 5 in all cultivars, with high prevalence in 'Ryuusei' and 'Benichiyo', and low prevalence in 'Hotaru' and 'Jukou' on January 12 to 20 (Table 2). No symptoms appeared in all the treatments until January 12. Disease occurrences in the treatments were significantly lower than that in the control groups, indicating thermotherapy was effective in control of downy mildew. Furthermore, hot water treatment and hot air + hot water treatment were more effective than hot air treatment.

No leaf burn occurred in hot air treatment and hot water treatment. Leaf burn occurred only under hot air + hot water treatment in all the cultivars, but its ratings were depend on cultivars (Table 3), with significantly higher prevalence in 'Jukou' and 'Ryuusei' as compared with 'Hotaru' and 'Benichiyo'. Severe leaf burns (more than index 3) did not occur in all the cultivars, and the leaf burn rating did increase from 5–12 January in all the cultivars. In addition, new leaf burn did not occur in all cultivars on January 24 and 30 (data not shown).

Fig. 1. *Chrysanthemum morifolium* with chrysanthemum downy mildew symptoms: **a** lower leaves turned yellowish-brown, **b** white powdery flora on the underside of leaves, **c** poor growth and dying of plants (cv. Shiragiri), **d** non-diseased plants (cv. Kawakaze).

Table 2. Control efficacy for chrysanthemum downy mildew with thermotherapy of chrysanthemum cuttings

Cultivars	Treatments	Accumulation of diseased plants (%)				No. of plants
		1/5	1/12	1/24	1/30	
Hotaru	Hot water ^z	0	0	0	0	27
	Hot air ^y	0	0	0	3.3	30
	Hot air + Hot water ^x	0	0	0	0	30
	Control	8.0	8.0	8.0	12.0	25
Jukou	Hot water	0	0	0	4.8	21
	Hot air	0	0	0	0	27
	Hot air + Hot water	0	0	3.8	3.8	26
	Control	4.0	12.0	12.0	12.0	25
Ryuusei	Hot water	0	0	0	0 a ^w	28
	Hot air	0	0	6.3	6.3 a	32
	Hot air + Hot water	0	0	0	0 a	32
	Control	3.1	59.4	59.4	59.4 b	32
Benitiyo	Hot water	0	0	0	0 α	18
	Hot air	0	0	37.5	37.5 β	32
	Hot air + Hot water	0	0	9.4	9.4 α	32
	Control	5.3	63.2	63.2	89.5 γ	19

^z Cuttings from infection mother plants were soaked in constant temperature water bath at 46°C for 3 min.

^y The mother plants were brought into the incubator; 20°C for 8 h, 35°C for 6 h and 15°C for 10 h between December 16 to 26.

^x Hot air + Hot water treatment: For plants given both thermotherapies, hot air treatment preceded hot water treatment, followed the methods described above.

^w The letters a-b and α-γ indicate treatments that differ significantly ($p < 0.05$) according to Ryan's multiple comparison test.

Table 3. Effect of hot air + hot water treatment on occurrence of leaf burn in chrysanthemum cuttings

Date	Cultivars	Leaf burn index ^z					Index ^y average
		0	1	2	3	4	
1/5	Hotaru	28	2	0	0	0	0.07 a ^x
	Benichiyo	25	7	0	0	0	0.22 ab
	Jukou	10	16	0	0	0	0.62 cd
	Ryuusei	13	18	1	0	0	0.63 cd
1/12	Hotaru	22	6	2	0	0	0.33 abc
	Benichiyo	18	7	7	0	0	0.66 bcde
	Jukou	5	8	13	0	0	1.31 ef
	Ryuusei	3	10	19	0	0	1.50 f

^z Leaf burn index: 0 = no leaf burn, 1 = slight brown spots in upper leaves, 2 = leaf burn occupied less than 10% of upper leaf areas, 3 = leaf burn occupied more than 10% of upper leaf areas or shoot tip was burnt, 4 = dying plants

^y Index average = (4A + 3B + 2C + D)/N; A, B, C and D are the numbers of plants on scale 4, 3, 2 and 1, respectively, and N is the total number of plants.

^x The letters a-f indicate cultivars that differ significantly ($p < 0.05$) according to Steel-Dwass multiple range test.

Control efficacy with fungicides

Results of fungicide trials in four cultivars are presented in Table 4. The disease severity was high (3.3–3.4) in the control through the experimental period. The level of disease control with azoxystrobin conferred the high

protection value in 'Benichiyo' (50.1) and 'Jukou' (30.4), but low in 'Hotaru' (17.8) and 'Ryuusei' (10.4) on April 25. Following that, manzeb conferred high protection in 'Benichiyo' (39.8) and 'Jukou' (35.6). No visible phytotoxicity was observed for any treatment. The disease

Table 4. Fungicide efficacy for chrysanthemum downy mildew among cultivars of chrysanthemum morifolium

Cultivars	Active ^z Ingredient	Disease severity ^y (Protective value ^x)						No. of Plants
		4/12		4/18		4/25		
Hotaru	Manzeb	2.2 abc ^w	(21.9)	2.5 bc	(19.3)	2.2 ab	(4.4)	25
	Tolfenpyrad	2.7 de	(2.7)	2.7 bc	(12.4)	1.9 ab	(15.4)	33
	Azoxystrobin	2.2 ab	(21.3)	2.1 ab	(30.3)	1.9 a	(17.9)	28
	Water	2.8 bcdef	—	3.1 cd	—	2.3 abc	—	27
Jukou	Manzeb	3.8 gh	(10.1)	3.0 cd	(21.1)	2.7 bcd	(35.6)	23
	Tolfenpyrad	3.9 gh	(0)	2.9 cd	(16.7)	3.3 d	(14.4)	23
	Azoxystrobin	2.7 bcd	(32.3)	2.2 ab	(37.1)	2.7 abcde	(31.0)	20
	Water	3.9 gh	—	3.5 de	—	3.9 g	—	24
Ryuusei	Manzeb	3.8 h	(0.5)	3.1 cd	(17.7)	3.0 cdef	(20.0)	26
	Tolfenpyrad	3.5 efgh	(9.4)	3.0 cd	(19.7)	3.5 efg	(6.7)	22
	Azoxystrobin	3.3 defg	(14.2)	2.8 cd	(24.6)	3.4 defg	(10.4)	32
	Water	3.9 h	—	3.7 e	—	3.8 fg	—	30
Benichiyo	Manzeb	2.9 cdef	(9.0)	2.2 ab	(26.0)	2.0 ab	(39.8)	16
	Tolfenpyrad ^v	—	—	—	—	—	—	—
	Azoxystrobin	1.6 a	(48.7)	1.6 ab	(46.6)	1.7 a	(50.2)	29
	Water	3.2 cdefgh	—	3.0 cd	—	3.3 defg	—	26
all cultivars	Manzeb	3.2 b'	(6.7)	2.7 b'	(17.9)	2.5 a'	(23.6)	90
	Tolfenpyrad	3.4 b'	(1.8)	2.9 b'	(13.9)	2.9 b'	(11.8)	68
	Azoxystrobin	2.5 a'	(28.6)	2.2 a'	(33.9)	2.4 a'	(27.3)	109
	Water	3.4 b'	—	3.3 c'	—	3.3 c'	—	106

^z Chrysanthemum plants were sprayed with fungicides three occasions at seven-day intervals between 5-19 April.

^y The average disease severity is based on a semi-quantitative classification of disease severity, to which we applied weighted averaging. The score ranges from 0 (low severity) to 5 (high severity). See the Materials and Methods section for more details.

^x The protective value is a semi-quantitative measure of the degree of protection provided by the fungicide. It ranges from 0% (no protection) to 100% (complete protection).

^w Different letters indicate significant difference ($p < 0.05$) between varieties by Steel-Dwass multiple range test.

^y The letters a-h and a'-c' indicate groups that differ significantly ($p < 0.05$) according to the Steel-Dwass multiple range test.

^z Disease severity of tolfenpyrad on Benichiyo was non-data due to the small number of samples.

severity of tolfenpyrad in 'Benichiyo' cannot be determined due to the small number of samples.

Discussion

Occurrence and severity of chrysanthemum downy mildew were significantly different among the cultivars, which indicated that some cultivars might have had resistance factors against the disease. Lower leaves were the most affected by the disease and turned yellowish brown, same as previously reported Sakaguchi et al. (1980). Moreover, symptoms between the middle and upper leaves, for certain cultivars, were observed in this survey.

Thermotherapy with hot water and hot air + hot water showed the high control efficacy (Table 2), which could produce the healthy seedlings from infected mother plants. Leaf burn occurred only in hot air + hot water treatments in this trial, and has been recorded for hot water treatments

of 42°C for 30 min, 45°C for 10 min and 48°C for 1 min (Hirayama 2015), and 46°C for 5 min and 48°C for 3 min (Yamagishi et al., 2016). The extent of leaf burn differed among cultivars for hot air + hot water treatments (Table 3), and hot water treatments (Yamagishi et al., 2016). These indicate that we need to select optimal combinations of time-temperature that are most adaptable to each cultivar for suppressing the occurrence of the disease and leaf burns.

In the fungicide efficacy trial, while azoxystrobin and manzeb were significantly higher compared with control in two of the four cultivars (Table 4), their protection value was at low level under conditions of severe disease. Accordingly, we need to evaluate efficacy of fungicides with different modes of action and before infection occurs.

Chrysanthemum downy mildew has recently appeared in some prefectures, and arrived impacted in Nara Prefecture in early 2017. This epidemiology establishes the need to

research fungicide efficacy and timing of application. We recommend the use of resistant cultivars and the early application of fungicides before disease symptoms appeared. In addition, thermotherapy with hot water is effective to suppress the disease for chrysanthemum seedlings and expected to control chrysanthemum white rust and downy mildew, which might contribute to decrease the cost of fungicide applications.

However, we need to solve the problem about the occurrence of leaf buns by hot water treatment and suggest the method to maintain water stable temperature for this treatment to promote the usage by growers. This is a first report to evaluate the occurrence of chrysanthemum downy mildew in many cultivars in the field, and its control by thermotherapy and fungicides.

References

- Catherine, G. and S. Regine (1994) *Crit. Rev. Plant Sci.* 13(1): 57–75.
- Hirayama, Y., S. Asano., N. Yamamoto, A. Kurata and K. Inda (2015) *Annu. Rep. Kansai Plant Prot.* (57): 144. (in Japanese, Abstr)
- Ito, S. (1936) *Mycologia flora of Japan.* 1: 217. (in Japanese)
- Kishi, K. (1998) *Noubunkyou.* pp. 571.
- MAFF (2017) URL: https://www.e-stat.go.jp/stat-search/files?page=1&layout=datalist&stat_infid=000031637614&lid=000001194863, Cited 1 December, 2017.
- Miyagi Prefecture (2017) URL: <http://www.pref.miyagi.jp/uplosed/attachment/612906.pdf>, Cited 17 October 2017.
- Okabe, M. and T. Baba (2011) *Journal of the Faculty of Agriculture, Shinshu Univ.* 47: 25–33. (in Japanese)
- Sakaguchi, S., T. Iwase, T. Nishino and Y. Nonaka (1980) *Kyusyu PL Prot Res.* 20: 70–72. (in Japanese)
- Sugimura, T., K. Okayama and S. Matsushima (1996) *Bull. Nara Agric. Expt. Sta.* 27: 39–43. (in Japanese)
- Uchida, T., S. Asari and T. Ashizawa (1979) *Annual Report of the Kanto-Tosan Plant Protection Society.* 26: 78–79. (in Japanese)
- Yamagishi, N., Y. Ishiyama and T. Shimizu (2016) *Annual Report of the Kanto-Tosan Plant Protection Society.* 63: 41–43. (in Japanese)